The role of protein in the sexual behaviour of males of Ceratitis capitata (Diptera: Tephritidae): mating success, copula duration and number of copulations

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ABSTRACT. In this paper, we investigated the influence of protein ingestion during the early adult phase on the sexual behavior of males of Ceratitis capitata (Wiedemann, 1824). The following parameters were evaluated: mating success (ability to be chosen by females), number of copulations, and copula duration. Experiments were carried out using a fifteen-year old laboratory lineage with the occasional introduction of wild flies. Two groups of adult males fed a high-protein diet during their larval phase were given either a high-protein diet (based on Brewer’s yeast, concentration = 6.5 g/100 ml) or a no-protein diet. Both groups of males were exposed to females fed either a high-protein diet or a no-protein diet and were subsequently evaluated for the parameters listed above. All experiments were conducted at 25°C, 70% R.H. and with a photoperiod of L12:D12. The number of copulations was the only parameter affected by adult diet. Males fed a high-protein diet and exposed to females fed a no-protein diet had a greater number of copulations compared with males fed a no-protein diet.

KEY WORDS. Fruit flies; insects; nutrition; sexual selection.

Organisms need a variety of nutrients to complete their life-cycle. Amongst these, proteins stand out as being able to influence several different processes such as development, maintenance and reproduction (Slansky & Sribner 1985). Previous research on the role of proteins has been conducted on Ceratitis capitata (Wiedemann, 1824), an important pest of fruits worldwide (Malavasi et al. 2000). Experiments with this fly revealed that protein deficiency during the larval phase causes delay to complete this life stage, reduction in adult emergence (Cangussu & Zucoloto 1997, Plácido-Silva et al. 2005), reduction in the size of the adult female and impaired ability to produce eggs (Cangussu & Zucoloto 1993, 1995). Additionally, tests conducted with laboratory populations showed that ingestion of proteins in the adult phase prolongs longevity (Plácido-Silva et al. 2006) and that protein deficiency negatively impacts egg production (Cangussu & Zucoloto 1997). Furthermore, protein consumption in the beginning of the adult stage is positively correlated with various aspects of the sexual behavior of wild and laboratory males, as for example participation in leks, rate of copulation and rate of female insemination (Papadopoulos et al. 1998, Taylor & Yuval 1999, Shelly et al. 2002).

The lekking behavior of C. capitata is a well-known phenomenon (Arita & Kaneshiro 1989, Hendrichs & Hendrichs 1990, Whittier et al. 1992). A lek is a gathering of males that delimit their territory and exude pheromones to attract females (Féron 1962, Prokopy & Hendrichs 1979, Arita & Kaneshiro 1989). Females discriminate between males in the lek, rejecting most advances and copulating only with the male they perceive as best fit (Whittier et al. 1992, 1994). Because mating success in this system is a function of female choice, it is believed that sexual selection is a determining factor in the evolution of male characteristics and male mating systems (Whittier et al. 1992, 1994, Norry et al. 1999).

Male reproductive success in C. capitata has been shown to correlate with different factors such as nutrition (see review in Yuval et al. 2007), body size (Churchill-Stanland et al. 1986, Orozco & Lopez 1993, Rodriguez et al. 2002) and copula duration. However, the lack of a general consensus on the subject and the fact that C. capitata is reared in massive quantities for the Sterile Insect Technique (SIT), a method of biological control (Knipling 1955), make new research in this area particularly relevant. With respect to the effects of a protein-rich diet in the adult phase on the reproductive success of C. capitata, a brief list of the main publications (Tab. I) brings about the main controversies and relevant facts.

The goal of the present study was to compare the reproductive success of males of C. capitata fed a protein-rich diet against the reproductive success of males fed a diet without proteins, in the beginning of the adult phase. Three main questions were asked: 1) Do males fed on a protein diet have a higher mating success than males deprived of protein? 2) Do males fed on a protein diet copulate for longer periods of time than males deprived of protein? 3) Do males fed on a protein diet copulate more than males deprived of protein?
MATERIAL AND METHODS

The flies used in this study came from a population of *C. capitata* frequently supplied with wild specimens since 1997, and maintained in the Laboratory of Nutritional Ecology, Instituto de Biología, Universidade Federal da Bahia. Population maintenance followed ZUCOLOTO (1987). Immature were fed a diet of soy flour and Brewer’s yeast (CARVALHO et al. 1998).

Two diets were used in the experiments. The first diet contained Brewer’s yeast as a source of protein (concentration = 6.5 g per 100 ml) and was named “P/P” (protein present). This yeast concentration had been previously established as adequate for larvae and adults of *C. capitata* (PLÁCIDO et al. 2006). The second diet contained no protein and was named “P/A” (protein absent). Both diets contained the following ingredients: sugar (11.0 g), agar-agar (2.0 g), citric acid (1.0 g) e nipagin (1.0 g).

The following general methodology was used to measure the parameters mating success, copula duration and number of copulations. First, all specimens were separated according to their sex 24 hours after emergence and were maintained isolated from each other; males and females were fed either the P/P or the P/A diet during the first four days after emergence, which corresponds to the period of sexual maturation.

Second, male individuals from each group were marked with non-toxic green or blue paint (Guache). On each replicate, group colors were alternated, as flies of the family Tephritidae have thoracic and wing marks that may have signaling function (BURK 1981, SIVINSKI et al. 2001).

Observations on mating behavior were conducted during six consecutive days, from 7:00 am to 3:00 pm. According to our personal observations, six days corresponds to the maximum period of time for the first set of copulations to happen in this population. During this period, individuals were fed water saturated with sugar *ad libitum*. In the end of each daily observation (7:00 am - 3:00 pm), males and females were separated and placed in different cages containing their original diets. The cages were cleaned periodically to avoid ingestion of proteins from excrements and dead individuals. The following controlled conditions were maintained throughout the experiments: 25°C temperature, 70% relative humidity and photoperiod of 12 hours.

In order to evaluate mating success, eight males (four from each group) were placed in a plastic cage (16x11x10 cm) together with four female virgins of either group. As soon as mating was spotted, the mating couple was carefully placed into a test tube until the end of the copulation. Couples were not re-introduced into their original cages. A total of 16 replicates (cage = replicate unit), eight for each female group, were conducted.

Copula duration was recorded for all mating couples observed in the mating success experiment. In order to ensure that manipulation of specimens did not interfere with the results, however, only copulations of 15 minutes or more were considered. For each group of males tested, 25 copulations were evaluated.
The role of protein in the sexual behaviour of males of *Ceratitis capitata*

Two tests were conducted in order to evaluate the number of copulations per male. The first test (“A”) consisted of exposing a male of the P/P or the P/A group to four female virgins (four days old) of the P/P group. The second test (“B”) consisted of exposing a male of the P/P or the P/A group to four female virgins of the P/A group. Mating couples were carefully placed into a test tube as soon as they were spotted, where they were kept until the end of the copulation. The male was then transferred to another cage containing four new female virgins, with conditions as before. A total of 19 males exposed to females on a P/P diet, and a total of 15 males exposed to females on a P/A diet were evaluated.

We used the non-parametric Mann-Whitney test to evaluate the results obtained for number of copulations and mating success; the unpaired t test (with the Welch correction when necessary) for results of copula duration, the Kolmogorov-Smirnov test to access whether the data fit a normal distribution, and the Bartlett test to ascertain the homoscedasticity of the data distribution. Statistical tests were performed with the help of the program GraphPad InStat_version 3.00, with α = 0.05. Bonferroni correction was used whenever necessary.

**RESULTS**

The results for the three parameters evaluated, i.e. mating success, copula duration and copulation numbers are presented in table II. With respect to the rate of mating success, males of both groups, P/P and P/A, had statistically similar performances, regardless of female diet. The copula duration was also statistically similar for both groups of males. With respect to the number of copulations, on the other hand, males of the P/P group copulated more often with females of the P/A group compared with males of the P/A group. This tendency was not observed when males of either group were exposed to females of the P/P group.

**DISCUSSION**

Previous studies using wild lineages of *C. capitata* have shown that ingestion of proteins in the adult phase enhances various aspects of male sexual behavior, as for example participation in leks, pheromone production, rate of copulation, and rate of sperm transfer (Yuval et al. 1998, Kaspi et al. 2000, Yuval et al. 2007). Contrasting results have been found when laboratory lineages are used, however (see Tab. I). For example, Papadopoulos et al. (1998) showed that wild adult males fed a protein-rich diet mature earlier and exude pheromones more frequently with respect to males deprived of protein, a result not confirmed in experiments using laboratory lineages. Furthermore, Shelly et al. (2002) were unable to verify the influence of protein ingestion on pheromone production in a laboratory population, a result that contrasts with that of Kaspi & Yuval (2000), who studied sterile males of a laboratory lineage.

The results obtained in this study do not corroborate previous findings that laboratory males fed a protein rich diet are more likely to copulate than males fed a protein-poor diet (Blay & Yuval 1997, Kaspi & Yuval 2000, Shelly et al. 2002). Other previous studies have also failed to demonstrate the effects of diet on the competitive abilities of laboratory males, either with each other or with wild males (Shelly & Kennedy 2002, Shelly & McInnis 2003, Shelly et al. 2006). According to Yuval et al. (2007), conflicting results from various studies reflect diverse methodologies, different parameters being evaluated and the different lineages used.

Another important fact to be noted in previous studies is that the diet given to the flies tested during the larval phase is seldom specified. The flies used in our experiments were fed a high protein diet. Consequently, the lack of observed effects of protein deprivation on the sexual behavior of adult males could be explained by the fact that protein reserves carried over from the larval phase can be utilized by the young adult, as demonstrated for other insects (Boggs 1981, Karlsson 1995). To a certain extent, protein and fat reserves in the beginning of the pupation period have been shown to correlate positively with the amount of yeast offered through the larval period (Nestel & Nemny-Lavy 2008); when in excess, however, protein gets deposited in the exocuticle of the pupa, becoming unavailable to the adult (Nestel et al. 2004). Further corroboration of the hypothesis that larval diet affects adult characteristics is the study of Kaspi et al. (2002), which demonstrates that a rich diet during the larval phase results in adult males that mature earlier. Additionally, a study by Maria do Carmo Plácido-Silva, Insti-

<table>
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<tr>
<th>Females</th>
<th>Mating success (number of males chosen)</th>
<th>Mann-Whitney test</th>
<th>Copula duration (minutes)</th>
<th>Unpaired T-test (with Welch correction)</th>
<th>Number of copulations/male</th>
<th>Mann-Whitney test</th>
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<tr>
<td></td>
<td>P/P</td>
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<tr>
<td>Males P/P</td>
<td>1.5 (0.0-3.0)</td>
<td>2.0 (1.0-3.0)</td>
<td>156.7 ± 67.3</td>
<td>145.9 ± 26.8</td>
<td>1.5 (1.0-4.0)</td>
<td>5.0 (3.0-8.0)*</td>
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<tr>
<td>Males P/A</td>
<td>2.5 (1.0-3.0)</td>
<td>2.0 (1.0-3.0)</td>
<td>163.6 ± 25.4</td>
<td>159.1 ± 40.1</td>
<td>4.0 (1.0-7.0)</td>
<td>3.0 (2.0-7.0)*</td>
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<td>P-value</td>
<td>0.1526</td>
<td>0.5573</td>
<td>0.7239</td>
<td>0.3701</td>
<td>0.0928</td>
<td>0.0493</td>
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tuto de Biologia, Universidade Federal da Bahia (unpublished data), showed that males fed a protein-rich diet during the larval phase and subsequently fed a low-protein diet in the beginning of the adult phase had higher rate of mating success when compared with males that received a protein-rich diet as larvae and adults. In contrast, males fed a protein-poor diet in the larval phase did not differ with respect to mating success in the adult phase, regardless of adult diet. These results highlight the importance of the larval diet on adult sexual behavior, and may in part explain the general consensus on the role of proteins in the adult phase of wild males. In fact, fruits (the natural diet of wild flies) are poorer in protein content when compared with yeast fed to populations in the laboratory. Consequently, adults from wild populations emerge with a shorter protein reserve and may therefore need a diet richer in proteins when compared with adults reared in the laboratory. A similar situation has been observed on the subject of female egg production in wild and laboratory populations of *C. capitata*. According to Yuval et al. (2007), wild females are anautogenous (require proteins in the adult life in order to produce eggs), whereas laboratory reared females are autogenous (do not require proteins in the adult life in order to produce eggs, even though protein consumption may increase egg production) with respect to proteins (Cangussu & Zucolotto 1993).

Most likely, the differing protein requirements between wild and laboratory lineages are due to the fact that the latter are adapted to the high protein contents of artificial diets which may exert selective pressure during the colonization of a laboratory population, as discussed by Meats et al. (2004).

As stated in the results section, copula duration resulted statistically independent from adult diet. Large standard deviations were also observed around the mean duration of copula in both groups. Similar variability in copulation duration was obtained in other studies. For example, Eberhard (1999) showed that copulation (counting only the events that led to storage of sperm in the spermatheca) lasted from 90 to 170 minutes within and across different lineages of fruit flies. Additionally, Paranhos et al. (2006) found that wild flies copulate for longer periods of time when compared with flies from laboratory lineages. Also, Field & Yuval (1999) showed that males and females with protein deficiency copulated for longer periods of time, despite individual variations in copulation length. The latter results were not corroborated by our data, which show no correlation between copula duration and diet. Shelly & Kennelly (2002) also failed to find a correlation between adult diet and copula duration. Prabhu et al. (2008), on the other hand, showed that males of another species, *Bactrocera tryoni* (Froggatt, 1897), copulated for longer periods of time when fed protein-rich diet, as opposed to males deprived of proteins. In conclusion, the various studies on the effects of diet on the reproductive behavior of fruit flies have proved difficult to determine the roles of age, nutrition and other factors on copula duration (Field et al. 1999).

Reproductive success in males and females is subject to different selective pressures, causing a conflict between the sexes (Anderson 1994, Birkhead & Parker 1997). A reproductively successful male copulates with a large number of females, whereas a female must be able to choose the best available mate in order to guarantee her reproductive success. Research on *C. capitata* has focused on female receptivity, i.e. the factors that contribute to her repeated copulations, as for example the duration of the first copula (Saul et al. 1988, Mossinson & Yuval 2003), nutritional status of the male (Blay & Yuval 1997, Shelly & Kennelly 2002), and male or female lineages (Shelly & Kennelly 2002, Mossinson & Yuval 2003, Vera et al. 2002, 2003). The parameters that interfere with the number of copulations a male can engage in, on the other hand, are seldom subject to research, despite their special relevance to control programs using the SIT. Indeed, the male potential to inseminate a large number of females is very important to the success of this technique. According to our study, males of the P/P group, when exposed to females of the P/A group, copulated more often than males of the P/A group exposed to the same group of females. When males of both groups were exposed to females of the P/P group, however, no significant differences in mating frequency was observed between groups. These results suggest that the number of times a male can mate on a given period of time is not dependent on his diet during the adult phase, but on the adult diet of the females he encounters. It appears as if the diet given to the female during her adult phase influences her ability to select her mates. Consequently, females deprived of protein accept well-nurtured males more often, either because these males show more vigorous displays or because the pheromones quality, or both. The females subjected to a protein rich diet, on the other hand, do not show this preference.

The reason for this difference in female behavior needs to be investigated by studies that take the nutritional levels of both sexes into consideration.

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**LITERATURE CITED**


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